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Short Communication

Satureja thymbra aqueous and ethanol extracts antibacterial activity

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Abstract

The concern toward using herbs around the world for the treatment of infectious diseases is increased during this century. Based on that, the following conducted experiment was performed to detect the effectiveness of using ethanol and aqueous extracts from Satureja thymbra L. (Lamiaceae) that is growing wild in Palestine. The bioactivity of both aqueous and ethanol extracts was tested against Pseudomonas aeruginosa (ATCC 27853), Staphylococcus aureus (ATCC 25923) and other two clinical isolates which are Klebsiella pneumoniae and Proteus mirabilis. Well susceptibility method and micro-broth dilution method were utilized to examine the antibacterial potential for both extract types under investigation. The obtained results showed that S. thymbra ethanol extract was better than aqueous one as it produced (10, 30, 16 and 12 mm) inhibition zones against K. pneumoniae, P. mirabilis, P. aeruginosa and S. aureus respectively. Meanwhile, S. thymbra aqueous extract had an impact on P. mirabilis with 20 mm zone of inhibition. The MIC results of the running experiment showed that ethanol extract exhibited a powerful inhibitory behavior as it prevented the growth of all tested microorganisms in a concentration range between 6.25 mg/ml and 25 mg/ml. Moreover, S. thymbra aqueous extract showed a moderate inhibition potential at 50 mg/ml MIC values to all examined bacterial isolates expect for K. pneumoniae clinical isolate. In conclusion, the acquired results confirmed the possibility of employing S. thymbra extracts in medicine and pharmaceutical industry of new drugs against some pathogenic bacteria.

Keywords: *Satureja thymbra*, plant extracts, antibacterial activity, medicinal plants, pathogenic bacteria.

Introduction

Folk medicine used the different extracts and essential oils from plant as natural therapies for thousands of years[1]. In many developing countries, medicinal plants and their derivatives play an important role in the primary health care as curative medication [2]. Therefore, researchers are focusing on medicinal plants, herbs and spices bioactivities. In this aspect, there are many reports on the antimicrobial activity of plant extracts and their essential oils that could serve as novel sources for antimicrobial agents against different microbes[1,3]. Extracts and essential oils of various species of edible medicinal plants, herbs, and spices compromise powerful natural bioactive agents. Among those agents, the secondary metabolites, hydrocarbons and oxygenated compounds which are responsible for odors and flavors of aromatic plants[3].

Species of the genus *Satureja* (Lamiaceae) are vastly spread in the Mediterranean region and Asia. They orderly present in warm, dry, rocky environments. *Satureja* contains of about 200 species, commonly aromatic herbs and used for curing of several diseases[4]. *Satureja thymbra* L. is one species of Lamiaceae family that is highly branched, ordinarily grey-puberulent dwarf shrub[5]. This plant species has a synchronous warming and an influence on the stimulation of the circulatory system. This makes it an excellent additive when it is used in low amounts in massage blends for arthritis and rheumatism. Furthermore, it can diminish joints and muscles pains[6].

In general, medicinal plants antimicrobial potential is related to the existence of active components, mostly attributable to isoprenes such as monoterpenes, sesquiterpenes and associated alcoholic compounds, in addition to other hydrocarbons and phenols[7,8]. In literature, scientist found that *S. thymbra* essential oils had a valid antimicrobial activity against several bacterial strains[9-11]. Therefore, the current study designed to estimate the antibacterial power of aqueous and ethanol extracts of *S. thymbra* that is growing wild in Palestine as most of the former studies concentrated on the biological activity of *S. thymbra* essential oils.

Materials and methods

Plant material: *Satureja thymbra* was collected from West Bank, Palestine and classified by Dr. Ghadeer Omar, Biology & Biotechnology Department, An-Najah National University, Palestine. The collected plant material for the antibacterial study was washed, dried, crushed into powder and kept dry at room temperature. A plant specimen was dried by pressing with chemical treatment. Then it was placed on herbarium sheet and given a voucher number (1365). The prepared sheet was deposited at An-Najah National University herbarium.

Extracts Preparation: The aqueous plant extract was prepared as, 10g of the crushed plant powder were macerated by soaking in 100ml warm sterile distilled water with frequent shaking for 7 days. Then the extracted solution was separated from the plant material by centrifugation at 5000rpm for 5min. The gained supernatant was evaporated by lyophilization. The lyophilized powder extracted from the studied plant species was solubilized in sterile distilled water to reach a final concentration equal to 100mg/ml. In the same way, the ethanol extract was prepared as the following, 10g of the crushed plant powder were macerated in 100ml of 70% ethanol for 7 days with frequent shaking. Then, the extracted solution was separated from the plant material by centrifugation at 5000 rpm for 5 min. Rotary evaporation was utilized to evaporate the resulting supernatant. For powder solubilization, 5% dimethyl sulfoxide (DMSO) was used as an alternative to water[12].

Bacterial Isolates: The *in vitro* antibacterial activity of *S. thymbra* aqueous and ethanol extracts was assessed against two bacterial isolates which were obtained from the American Type Culture Collection (ATCC). Those isolates are *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 27853). Additionally, two clinical isolates which are *Klebsiella pneumoniae* and *Proteus mirabilis* that were obtained from a local hospital (Rafidia Hospital, Nablus, Palestine), and their identification was confirmed by API20 (bioM'erieux, France).

Antibacterial Activity Assay: Well susceptibility test was conducted to analyze the antibacterial potential of the two prepared extracts[13]. The examined bacteria were grown on nutrient agar plates to reach a log phase in the bacterial growth curve. Then the numbers of bacteria were quantified by comparing them with 0.5 McFarland standards (1.5×10^8 CFU/ml). For susceptibility test, Mueller Hinton agar plates were inoculated by swabbing their surfaces with the adjusted bacteria. To do that, the plate rotated 60º each time to distribute the inoculums evenly. After that, 25µl of 100mg/ml from each of the studied plant extract were filled in 6 mm wells that was previously prepared in the inoculated agar plates. To ensure that the loaded extract diffused into the agar, all prepared plates were standing at room temperature for 30min before their incubation at 37°C for 18h. After incubation, the inhibition of bacterial growth was estimated by the measurement of the nearest mm of the resulting inhibitory zones (IZD). The test was carried out in triplicates and a broad spectrum antibiotic Gentamicin (G) used as positive control.

For precise evaluation of the minimum inhibitory concentration (MIC) for the two studied plant extracts, the standard micro-broth dilution test was employed[14]. The test began by applying two fold serial dilution for the two extracts in a liquid medium. Then, duplicates of each dilution started from 50mg/ml and ended up with 0.098 mg/ml were inoculated with 1×10^5 CFU/ml of the adjusted bacteria. For control preparation, two duplicate wells were not inoculated with bacteria and considered as negative controls and other two duplicate wells were inoculated with tested bacteria to be used as positive controls. After that, all microwell plates were incubated at 37°C for 18h. At the end of incubation, the bacterial turbidities in all wells were visualized in order to estimate the lowest extract concentration that inhibited the growth of the examined bacteria which also known as MIC.

Results and discussion

Through the past years, the concern for natural medicine in the developed societies has been increasing primarily in medical sectors[15]. The emergence of antibiotic resistant bacterial isolates is a critical public health challenge. Among those bacterial isolates; *Klebsiella pneumoniae*, *P. mirabilis*, *P. aeruginosa* and *S. aureus* which are considered to be one of the most important causes of some infections[15]. In this aspect, screening for an alternative to the ordinary antimicrobial agents like plants and their derivatives is proposed specially in Palestine. Therefore, the current study was performed to evaluate the antibacterial potential of *S .thymbra* that is growing wild in Palestine. The antibacterial estimation by well diffusion assay of both aqueous and ethanol extracts obtained from *S .thymbra* showed that they posses potent antibacterial activity against the bacterial isolates under study (Figure-1). It was obviously noticed that *S. thymbra* ethanol extract was better than aqueous one as it produced (10, 30, 16 and 12mm) zones of inhibition against *K. pneumoniae*, *P. mirabilis*, *P. aeruginosa* and *S. aureus* respectively. Meanwhile, *S. thymbra* aqueous extract acted effectively against *P. mirabilis* with 20 mm zone of inhibition. On top of that, the recorded antibacterial activity for the two prepared extracts against this clinical isolate of *P. mirabilis* was higher than the wide spectrum antibiotic Gentamycin.

The antibacterial efficacy of the studied extracts was quantitatively evaluated by the determination of their MIC values against the four examined bacteria (Figure-2). The MIC results of the running experiment showed that ethanol extract exhibited an effective inhibitory behavior as it inhibited the growth of all examined microorganisms in a concentration range between 6.25mg/ml and 25mg/ml. Moreover, *S. thymbra* aqueous extract showed a moderate inhibition power at 50 mg/ml MIC values to all examined bacterial isolates expect for *K. pneumoniae* clinical isolate. This *K. pneumoniae* isolate not inhibited by *S. thymbra* aqueous extract at the examined concentrations. The findings in the running research are compatible with others obtained in a number of the previous investigations to a particular degree taking into consideration the antibacterial effect of the essential oils of *S. thymbra*[4,9,16-19]. In this regards, many studies regarding the biological activity of plant essential oils confirmed that those oils possess a considerable antibacterial activity including *S. thymbra*[20]. According to the previous researches, the modes of action of some essential oils against pathogenic or non-pathogenic bacteria were related to several mechanisms rather than to a specified one[21]. Moreover, previous investigations of essential oil chemical composition from *S. thymbra* indicated that it

included carvacrol and thymol as major components[22,23]. Apparently, the chemical composition of *S. thymbra* oils provided evidence that there is a direct relation between the high antibacterial activity of this plant species and its high phenolic content[19]. Besides that, a study concerning the effect of both aqueous and ethanol extracts from wild *S. thymbra* in Palestine showed a moderate antibacterial behavior against multidrug resistant *Escherichia coli*[24].

The current research outcomes clearly indicated that the antibacterial activity varies with the extract type. This antibacterial variation of screened plant extracts could be explained by the fact that different solvents have various degrees of solubility according to the type of phyto-constituents. Accordingly, the obtained results revealed the necessity for further phytochemical and pharmacological studies which are promoted to purify and characterize the active ingredients of the studied plant species.

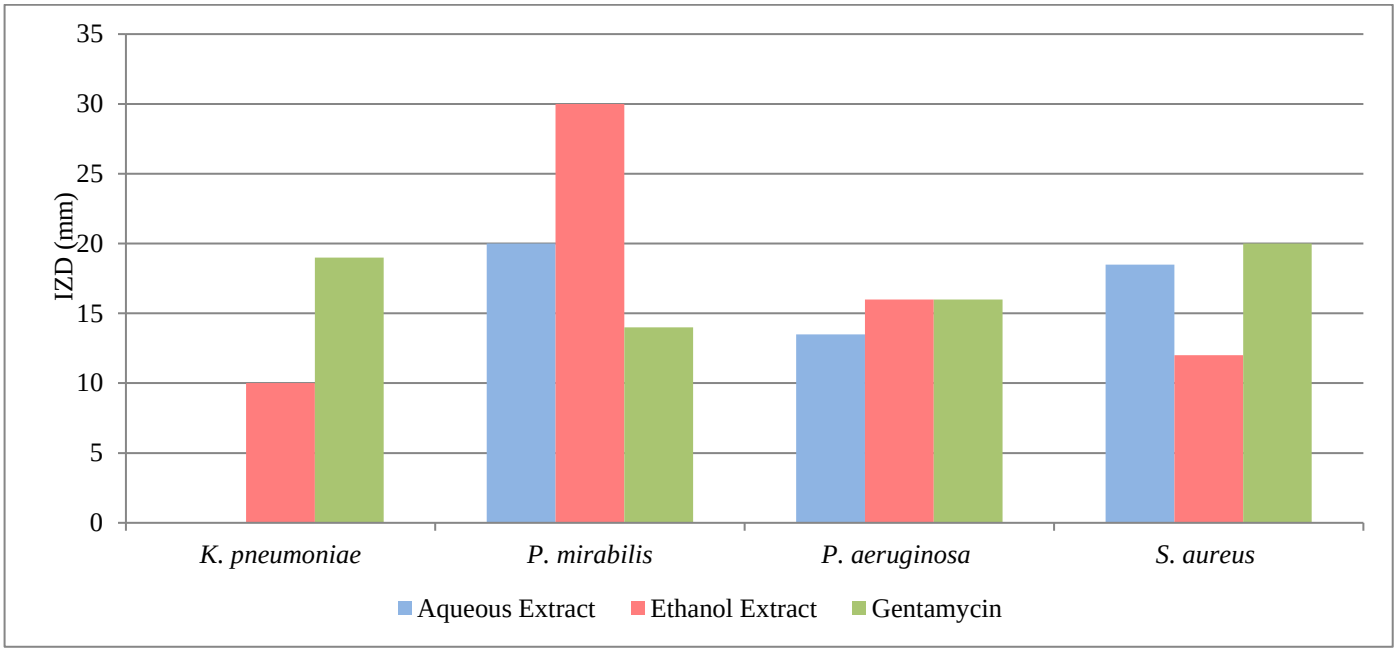

Figure-1: Antibacterial effect of *S. thymbra* aqueous and ethanol extracts by agar well diffusion assay; (IZD) inhibition zone diameters.

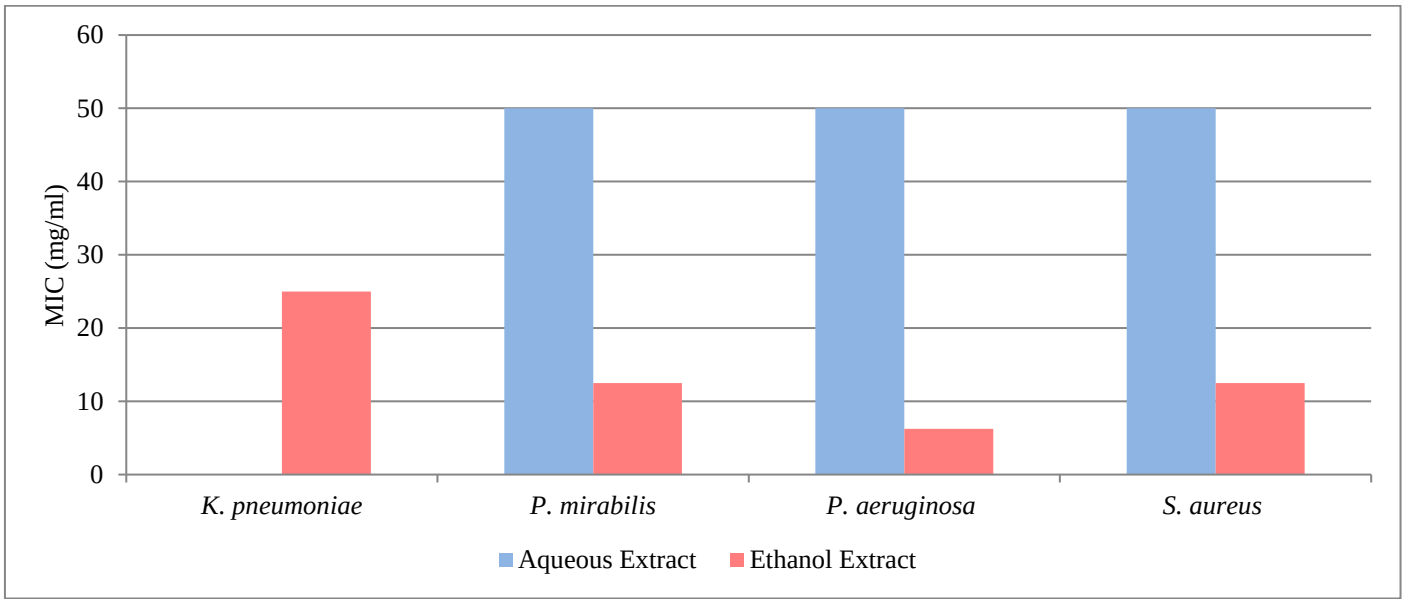

Figure-2: Antibacterial effect of *S. thymbra* aqueous and ethanol extracts against four examined bacteria by micro-dilution assay; (MIC) minimum inhibitory concentration (mg/ml).

Conclusion

This research confirms the folkloric expectation of *S. thymbra* as antibacterial and therapeutic agent, proving that the folkloric medicinal plants are a remarkable sources for diverse natural products in curing common infectious diseases caused by many microbes including bacteria.

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